ing insulin sensitivity. Recently, adiponectin has also been found to regulate immune responses and inflammation. Adiponectin is found in OA joints but its role in the pathogenesis of OA and in cartilage metabolism is not clear. In the present study, we investigated the relation of circulating adiponectin and biomarkers of cartilage degradation (COMP and MMP-3) in patients with OA, and the effects of adiponectin on human OA cartilage.
Methods: Blood samples were collected from 38 male OA patients (BMI 29.5±0.8 kg/m^2) undergoing knee replacement surgery because of severe OA, and adiponectin, COMP and MMP-3 concentrations were measured by immunoassay. Cartilage samples collected from OA patients under total knee arthroplasty were placed in tissue culture and exposed to adiponectin.
Results: Plasma adiponectin (2.5±0.2 μg/ml) correlated positively with serum COMP (r=0.55, p=0.001) and plasma MMP-3 (r=0.34, p=0.046). In tissue culture experiments, adiponectin increased the expression of iNOS, and production of nitric oxide, interleukin-6 (IL-6) and MMP-3 in human OA cartilage. The effects of adiponectin of NO and IL-6 production were mediated through MAP kinases Erk1/2, p38 and JNK, and p38 pathway was involved in the adiponectin-induced MMP-3 production.
Conclusions: Circulating adiponectin concentrations correlated positively with the measured biomarkers of cartilage degradation, i.e. COMP and MMP-3 in male OA patients; and adiponectin was found to increase the production of catabolic/proinflammatory mediators MMP-3, nitric oxide and IL-6 in human OA cartilage. The findings introduce adiponectin as a catabolic factor in OA.

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TOLL-LIKE RECEPTORS ACTIVATE THE ESE-1 PROMOTER IN CHONDROCYTES BY A MECHANISM PARTIALLY DEPENDENT UPON NF-κB

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Purpose: Toll-like receptor (TLR)2 is increased in OA cartilage and TLR2 ligands trigger catabolic responses in chondrocytes, including MMP release. We previously showed that Epithelial Specific ETS (ESE)-1 expression is induced by inflammatory factors, including LPS, in non-epithelial tissues and that it acts as a primary mediator of inflammatory responses. In addition, ESE-1 levels are higher in OA chondrocytes, where it mediates IL-1-induced *COL2A1* promoter repression, suggesting its involvement in cartilage catabolism. In this study, we aimed to investigate the role of TLR2 in regulating ESE-1 gene expression in chondrocytes.
Methods: Immortalized human C28/I2 chondrocytes were transfected with TLR2, TLR-4 and MyD88 expression vectors, and ESE-1 messenger RNA (mRNA) was analyzed by real time RT-PCR. Transactivation by TLR2 and TLR4 was analyzed using the human *ESE1* promoter sequences spanning -1541 to +29 cloned into a luciferase reporter (pXP2/ESE1wt) in co-transfection experiments with p50, p65 and MyD88 expression vectors. To examine the role of NF-κB in *ESE1* transactivation by TLR2, the pXP2/ESE1mut with a mutation in the NF-κB site was utilized in co-transfections. The specific NF-κB inhibitors SN50 and caffeic acid phenethyl ester (CAPE), both of which inhibit translocation of the NF-κB complex into the nucleus, were used to further confirm NF-κB involvement on *ESE1* promoter activation and endogenous mRNA levels.
Results: ESE-1 mRNA was induced by TLR2 alone or together with MyD88, and CAPE decreased this induction in C28/I2 cells. In luciferase reporter assays, TLR2 overexpression, alone or together with MyD88, activated the pXP2/ESE1wt promoter, whereas TLR4 required MyD88 for the activation and the pXP2/ESE1mut was less responsive to TLR2 without or with the MyD88 adaptor. TLR-2 alone gave a modest increase in ESE-1 promoter activity, while TLR2 cooperated with NF-κB p65/p50 in transactivating the ESE-1 promoter activity, However, TLR4 required its adaptor MyD88 to cooperate with p65 and p50 in transactivating the ESE-1 promoter activity. The addition of an NF-κB inhibitors, SN50 or CAPE, partially blocked the TLR2-induced *ESE1* promoter activity alone or together with MyD88.
Conclusions: Our previous findings that ESE-1 is induced by inflammatory factors, including LPS, a TLR4 ligand, prompted us to ask whether ESE-1 participates in cartilage catabolic processes induced by TLRs. These receptors have been detected in OA cartilage, where they are proposed to augment joint destruction. Indeed, TLR2 levels are higher in OA cartilage and its ligands induce chondrocyte catabolic responses, including MMP release and type II collagen degradation. Here, we show that the *ESE1* promoter activity and endogenous ESE1 mRNA were up-regulated by TLR2 and that this activation partially relies on the translocation of the NF-κB family member p50 and p65 to the nucleus. Given the role of ESE-1 as a key regulator of responses to inflammatory mediators, our results suggest that ESE-1 may participate in the catabolic responses triggered by TLR2 or TLR4 in OA cartilage.

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EXPRESSION OF MOLECULAR MARKERS INVOLVED IN ENDOCHONDRAL OSSIFICATION DURING OSTEOARTHRITIS IN AN ANIMAL MODEL

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Purpose: To investigate expression of molecular markers involved in endochondral ossification during osteoarthritis (OA) in an animal model. The expression of Wnt4, Wnt5a and Wnt5b, and the activity of β catenin were correlated with the expression of p57^{Kip2}, p107 and cartilage markers (Sox9, Aggrecan and Collagen II, Collagen X); data were also correlated with the expression of proinflammatory cytokines and apoptosis at different stages of OA.
Methods: Wistar male rats were sacrificed at 5, 10 and 20 days following a partial menisectomy in order to induce experimental OA. As normal control we used knees from animals without exercise and surgery. Histological, immunohistochemistry and *in situ* hybridization analyses were performed for Safranin-O fast green stain, IL1β, IFNγ, TNFα, β catenina, p107, p57^{kip2}, aggrecan, Sox9, collagen type II, collagen type X, Wnt4, Wnt5a and Wnt5b. A TUNEL test was used to assess programmed cell death.
Results: During OA the first molecular changes were observed as soon as 5 days after surgery, even though no macroscopically changes were observed. The expression of Sox9, aggrecan and collagen type II was down-regulated indicating loss of articular chondrocyte phenotype. These results coincided with up-regulation of p57^{kip2} and p107, an increase of cellular death and expression of collagen type X, indicative of hypertrophy of articular cartilage as occurs during endochondral ossification. Also TNFα, which is an important catabolic cytokine involved in the initiation and progression of articular cartilage destruction, was up-regulated at 5 days post-surgery, while IL1β and TNFα were up-regulated at 10 days post-surgery in cartilage damaged, as compared with the controls. All these molecular changes are associated with molecular changes of key regulators of endochondral ossification, belonging to Wnt family. The promoter of hypertrophy of cartilage Wnt4 was up-regulated coinciding with the onset of